

**Biological Nitrogen Removal through Nitritation Coupled with
Thiosulfate-Driven Denitritation**

(Supplementary Information)

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Table S1. Composition of stock nutrient solution for both nitrifying sludge^a and AnUSB reactor.

Component	Concentration (g/L)	Component	Concentration (g/L)
NH ₄ Cl	18.45	FeCl ₃ ·6H ₂ O	2
K ₂ HPO ₄	1.92	H ₃ BO ₃	0.2
KH ₂ PO ₄	0.72	CuSO ₄	0.05
MgCl ₂ ·6H ₂ O	8.32	KI	0.08
CaCl ₂	5.2	MnSO ₄ ·4H ₂ O	0.25
NaHCO ₃	62.4	ZnSO ₄ ·7H ₂ O	0.15
		CoCl ₂ ·6H ₂ O	0.2

^aOrganic carbon (as glucose) was additionally dosed to the nitrifying sludge, resulting in an influent COD concentration of 480 mg COD/L.

Table S2. Conditions for the nitrifying sludge cultivation.

Effective reactor volume (L)	2.4
Exchange ratio	0.5
Temperature (°C)	23 ± 1
pH	7.5~8.0
HRT (hrs)	8
DO concentration (mg/L)	2~3
Influent NH ₄ ⁺ concentration (mg N/L)	240
Influent COD concentration (mg COD/L)	480
MLVSS concentration (mg/L)	3200

Table S3. Conditions (i.e. pH, initial NO_2^- and $\text{S}_2\text{O}_3^{2-}$ concentrations) for the 8 batch reactors and 8 control reactors (without $\text{S}_2\text{O}_3^{2-}$ as the electron donor) in Batch Test II – biomass-specific denitrification activities under different initial NO_2^- concentrations, pH and FNA concentrations.

		pH	NO_2^- conc. (mg N/L)	$\text{S}_2\text{O}_3^{2-}$ conc. (mg S/L)
Batch Reactors	1	7.5	30	360
	2	7.5	60	
	3	7.5	90	
	4	7.5	120	
	5	6.0	60	
	6	7.0	60	
	7	8.0	60	
	8	9.0	60	
Control Reactors	1	7.5	30	0
	2	7.5	60	
	3	7.5	90	
	4	7.5	120	
	5	6.0	60	
	6	7.0	60	
	7	8.0	60	
	8	9.0	60	

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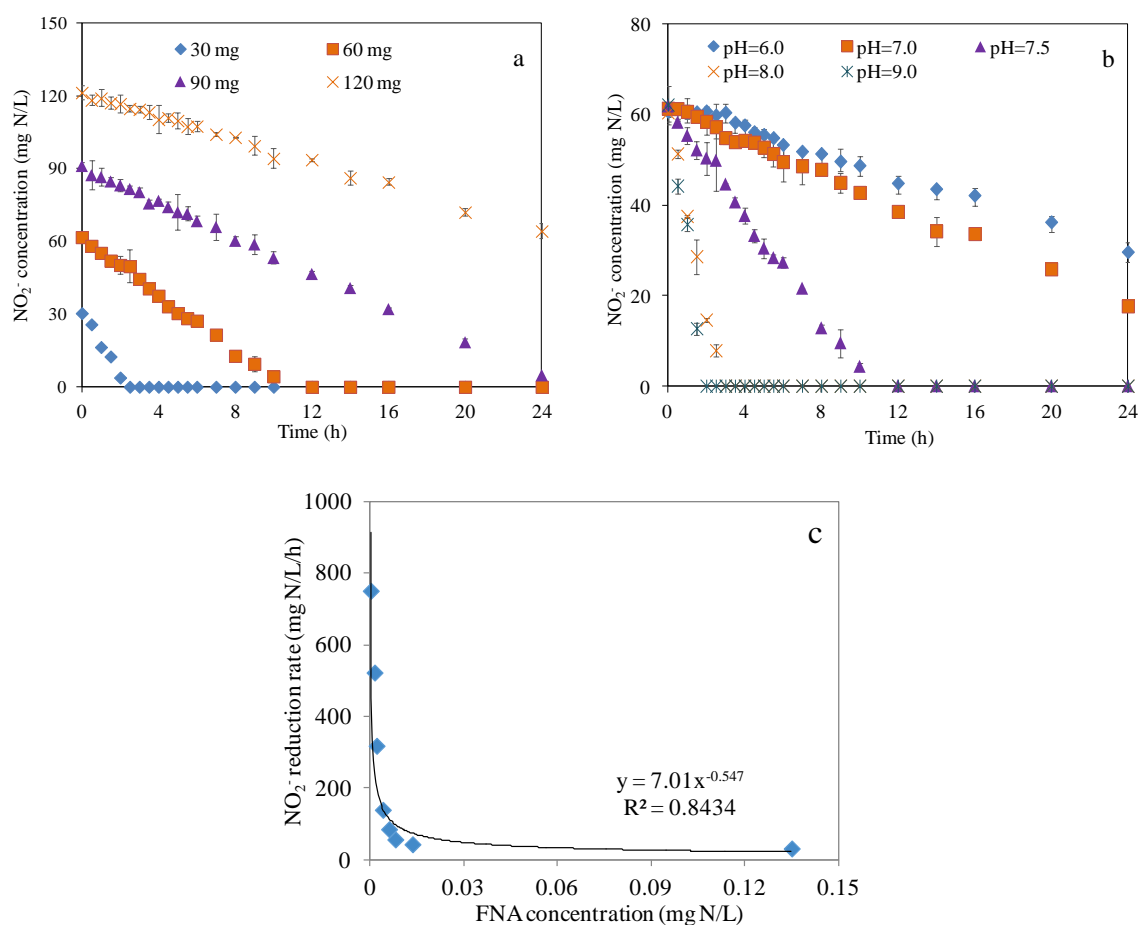
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Table S4. Primer of the DNA amplification for NSBR and AnUSB reactor at the beginning and end of operation.

Barcode Sequence	Primer
	(V1-V3)
ATGCTACGTC	8F: 5'-AGAGTTTGATCCTGGCTCAG-3'
	533R: 5'-TTACCGCGGCTGCTGGCAC-3'

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31 **Figure S1.** Batch Test II results - biomass-specific denitrification activities under
 32 different initial NO_2^- concentrations, pH and FNA concentrations: (a) Profile of nitrite
 33 for Batch Reactors 1 to 4 (under different initial NO_2^- concentrations) in Batch Test II;
 34 (b) Profile of nitrite for Batch Reactors 5 to 8 (under different pH) in Batch Test II; (c)
 35 relationship between NO_2^- reduction rates and initial FNA concentrations in each
 36 reactor of Batch Test II.